

# PATENT ABSTRACTS OF JAPAN

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(54) ENHANCER FOR CALCIUM INCLUDING TISSUE AND APPLICATION OF THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To provide an agent having an enhancing function to the tissue including calcium, safely usable and to provide application of the agent.

SOLUTION: This enhancing agent for the calcium including tissue includes one or two or more kind of compounds selected from flavones, flavonols, fravanones, fravanonols, anthocyanidins, flavanols, chalcones or aurones or their precursors.

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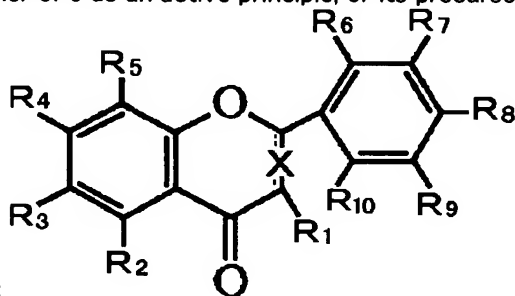
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CLAIMS

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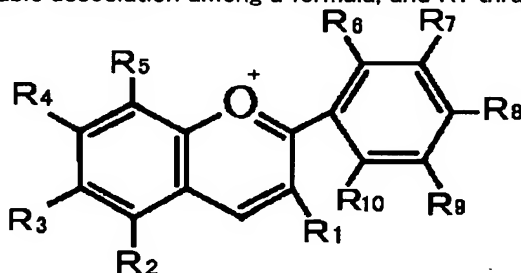
[Claim(s)]

[Claim 1] The calcium organization reinforcement characterized for the matter which has the basic structure expressed with a general formula 1 thru/or either of 5 as an active principle, or its precursor by one sort or containing two or more sorts.



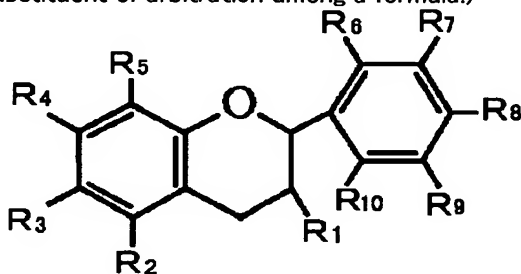
[Formula 1] General formula 1 :

(X expresses single bond or double association among a formula, and R1 thru/or R10 express the substituent of arbitration.)



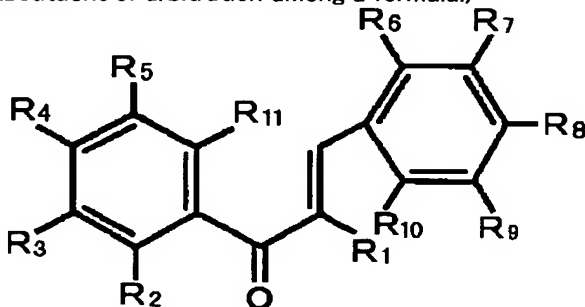
[Formula 2] General formula 2 :

(R1 thru/or R10 express the substituent of arbitration among a formula.)



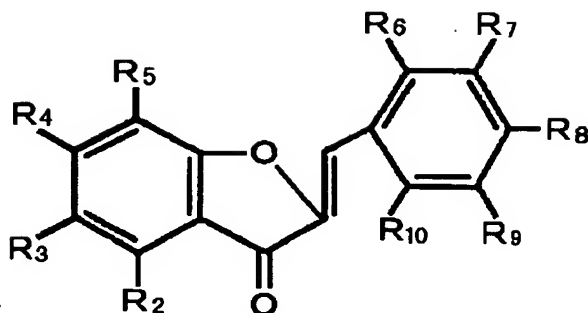
[Formula 3] General formula 3 :

(R1 thru/or R10 express the substituent of arbitration among a formula.)



[Formula 4] General formula 4 :

(R1 thru/or R11 express the substituent of arbitration among a formula.)



[Formula 5] General formula 5 :

(R2 thru/or R10 express the substituent of arbitration among a formula.)

[Claim 2] The \*\* calcium organization reinforcement according to claim 1 with which the matter which has the basic structure expressed with a general formula 1 thru/or 5, or its precursor is characterized by being flavones, flavonols, flavanones, FURABANO Norians, anthocyanidins, flavanols, chalcones and/or Orlon, or a precursor of those.

[Claim 3] The \*\* calcium organization reinforcement according to claim 2 with which flavones, flavonols, flavanones, FURABANO Norians, anthocyanidins, flavanols, chalcones, and/or Orlon are characterized by being an aglycon, a glycoside, a polymer, and/or a derivative.

[Claim 4] The \*\* calcium organization reinforcement according to claim 2 or 3 whose flavonols are kaempferol or quercetine.

[Claim 5] The \*\* calcium organization reinforcement according to claim 2 or 3 whose flavanones are the hesperetin or a naringenin.

[Claim 6] The \*\* calcium organization reinforcement according to claim 2 or 3 anthocyanidins or whose precursor of those is cyanidin or a pro anthocyanidin.

[Claim 7] The \*\* calcium organization reinforcement according to claim 2 or 3 whose flavanols are catechins.

[Claim 8] A \*\* calcium organization reinforcement given in claim 1 thru/or any of 7 they are. [ which furthermore comes to contain isoflavone as an active principle ]

[Claim 9] The \*\* calcium organization reinforcement according to claim 8 whose isoflavone is ipriflavone or soybean isoflavone.

[Claim 10] The \*\* calcium organization reinforcement according to claim 8 or 9 whose isoflavone is an aglycon, a glycoside, a polymer, and/or a derivative and which comes out and is characterized by a certain thing.

[Claim 11] Furthermore, a \*\* calcium organization reinforcement given in claim 1 thru/or any of 10 containing one sort chosen from a mineral, the matter which has a mineral absorption promotion operation, an oligosaccharide, and a vitamin, or two sorts or more they are.

[Claim 12] The \*\* calcium organization reinforcement according to claim 11 with which a mineral is characterized by being a lime compound and/or a magnesium compound.

[Claim 13] The \*\* calcium organization reinforcement according to claim 11 or 12 with which the matter which has a mineral absorption promotion operation is characterized by being a casein phospho peptide and/or an oligosaccharide.

[Claim 14] The \*\* calcium organization reinforcement according to claim 11 to 13 with which an oligosaccharide is characterized by being fructo oligosaccharide, isomalt oligosaccharide, xylo oligosaccharide, RAKUTO sucrose, galacto-oligosaccharide, alpha, and alpha-trehalose and/or alpha, and beta-trehalose.

[Claim 15] The \*\* calcium organization reinforcement according to claim 11 to 14 with which a vitamin is characterized by being vitamin D, a vitamin K, L-ascorbic acid, and/or its derivative.

[Claim 16] The ingesta, cosmetics, or drugs given in any [ claim 1 thru/or ] of 15 they are which comes to blend a \*\* calcium organization reinforcement.

[Claim 17] Ingesta according to claim 16 characterized by ingesta being health food.

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[Translation done.]

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the application at the \*\* calcium organization reinforcement which can be used for making the amount of calcium in \*\* calcium organizations, such as an organization of a bone or a gear tooth, increase, and a list.

[0002]

[Description of the Prior Art] Generally, the embrittlement of \*\* calcium organizations, such as a bone and a gear tooth, happens with aging. In the osseous tissue, osteogenesis and osteoclasts are performed continuously, and when young, osteogenesis and osteoclasts can usually be balanced. However, the balance of osteogenesis and osteoclasts collapses according to various causes, such as imbalance of the hormone accompanying an extreme diet and extreme aging, and it inclines to osteoclasts. If this condition continues for a long time, the calcium which is an important constituent from \*\* calcium organizations, such as a bone and a gear tooth, will decrease, and it will become easy to produce diseases of tooth, such as bone diseases, such as osteoporosis, fracture, and low back pain, or a cavity, and gum disease.

[0003] In the \*\* calcium organization, calcium in the living body exists as a solid state with the gestalt of calcium phosphate salts, such as hydroxyapatite, forms a bone and a gear tooth, and it not only bears the role which strengthens those organizations, but has become the source of supply of calcium ion required for the body. It is said that the calcium intake of our country is liable to insufficient, and the various health ingesta which strengthened calcium are marketed. However, when good effectiveness cannot be desired from the point of absorption efficiency only by taking in calcium and it takes in too much, there is a danger of lapsing into high calcium plasma, and the intake approach of calcium takes prudence. Therefore, in order to make \*\* calcium organizations, such as a bone and a gear tooth, do the deposition of the calcium, calcium moderate as a nutrient is taken in, and in order to improve absorption and a metabolic turnover further, intake of other minerals, such as magnesium, is recommended. However, the effectiveness is not enough. Moreover, in addition to said mineral, prescribing oily vitamins and hormone drugs, such as vitamin D, calcitonin pharmaceutical preparation, an estrogen preparation, myotropic hormone pharmaceutical preparation, and bis-phosphonate, for the patient is also performed. Although this approach is more effective than only taking in a mineral, when using an oil solubility vitamin compound and a hormone drug, it is in the situation which cannot be said as the approach which that administration schedule is complicated, and fear of the side effect by over-medication etc. also has it, and it not necessarily satisfies.

[0004]

[Problem(s) to be Solved by the Invention] This invention is nonpoisonous and makes it a technical problem to offer the application to ingesta, cosmetics, drugs, etc. which come to blend the high \*\* calcium organization reinforcement and this high of insurance and the operation effectiveness.

[0005]

[Means for Solving the Problem] The matter which has the basic structure expressed with the general formula 1 mentioned later also unexpectedly thru/or either of 5 when this invention person etc. searches wholeheartedly paying attention to a vegetable component, or its precursor (henceforth) also describing it as a general formula 1 thru/or the matter of 5 on these specifications — it is — the calcium metabolism of an animal was guided in the direction of the calcification, the \*\* calcium organization was made to do the deposition of the calcium, the organization was strengthened, and it discovered making bone weight increase as a result. Furthermore, when using isoflavone with a general formula 1 thru/or the matter of 5, the calcium metabolism of an animal was guided in the direction of the calcification, the \*\* calcium organization was made to do the deposition of the calcium, the organization was strengthened, and it discovered making bone weight increase as a result, and resulted in this invention.

[0006] That is, this invention offers the \*\* calcium organization reinforcement which contains one sort or two sorts or more for a general formula 1 thru/or the matter of 5, for example, flavones, flavonols, flavanones, FURABANO Norians, anthocyanidins, flavanols, chalcones, Orlon, or the precursor of those as an active principle.

[0007] Furthermore, this invention offers the \*\* calcium organization reinforcement which contains isoflavone with the above-mentioned matter.

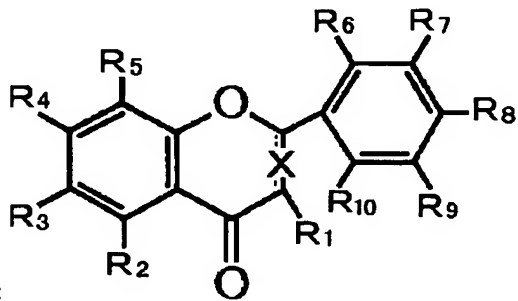
[0008] Furthermore, this invention offers the application to the ingesta which come to blend said \*\* calcium organization reinforcement, cosmetics, drugs, etc.

[0009]

[Embodiment of the Invention] The general formula 1 used by this invention for explaining the gestalt of implementation of this invention thru/or the matter of 5 are matter which belongs to the flavonoid widely distributed as a vegetable component originally. The matter which has the basic structure of a general formula 1 Flavones and flavonols It is what is called flavanones and FURABANO Norians. For example, the flavones characterized by for R1 being a hydrogen atom and X being double association R1 is a hydroxy group (or what is \*\*\*\*\* (ed) by a certain saccharide is included). And the flavonols characterized by X being double association and R1 are hydrogen atoms. And it is called FURABANO Norians characterized by for the flavanones characterized by X being single bond and R1 being hydroxy groups (or what \*\*\*\*\* (ed) by a certain saccharide being included),

and X being single bond. In this invention, the matter shown in Table 1 as matter which has the basic structure of a general formula 1 can be used. In addition, the glucose by which a hydrogen atom and OH were \*\*\*\*\* (ed) for H among Table 1, and a methoxy group and OGlu were \*\*\*\*\* (ed) for a hydroxy group and OCH3, the rhamnose by which ORha was \*\*\*\*\* (ed), and ORut show the \*\*\*\*\* (ed) rutinose.

[0010]



[Formula 6] General formula 1 :

(X shows single bond or double association among a formula, and R1 thru/or R10 show the substituent of arbitration.)

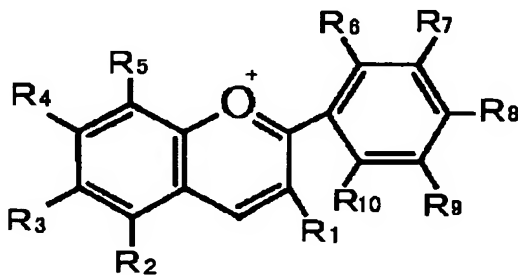
[0011]

[Table 1]

|        | 名称        | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub>   | R <sub>5</sub> | R <sub>6</sub> | R <sub>7</sub>   | R <sub>8</sub>   | R <sub>9</sub> | R <sub>10</sub> | X    |
|--------|-----------|----------------|----------------|----------------|------------------|----------------|----------------|------------------|------------------|----------------|-----------------|------|
| フラボン   | フラボン      | H              | H              | H              | H                | H              | H              | H                | H                | H              | H               | 2重結合 |
|        | クリシン      | H              | OH             | H              | OH               | H              | H              | H                | H                | H              | H               | 2重結合 |
|        | アピゲニン     | H              | OH             | H              | OH               | H              | H              | H                | OH               | H              | H               | 2重結合 |
|        | ルテオリン     | H              | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 2重結合 |
|        | アカセチン     | H              | OH             | H              | OH               | H              | H              | H                | OCH <sub>3</sub> | H              | H               | 2重結合 |
|        | リナリン      | H              | OH             | H              | ORut             | H              | H              | H                | OCH <sub>3</sub> | H              | H               | 2重結合 |
| フラボノール | フラボノール    | OH             | H              | H              | H                | H              | H              | H                | H                | H              | H               | 2重結合 |
|        | ケンフェロール   | OH             | OH             | H              | OH               | H              | H              | H                | OH               | H              | H               | 2重結合 |
|        | ケルセチン     | OH             | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 2重結合 |
|        | モリン       | OH             | OH             | H              | OH               | H              | OH             | H                | OH               | H              | H               | 2重結合 |
|        | ミリセチン     | OH             | OH             | H              | OH               | H              | H              | OH               | OH               | OH             | H               | 2重結合 |
|        | ケルセタゲチン   | OH             | OH             | OH             | OH               | H              | H              | OH               | OH               | H              | H               | 2重結合 |
|        | イソラムネチン   | OH             | OH             | H              | OH               | H              | H              | OCH <sub>3</sub> | OH               | H              | H               | 2重結合 |
|        | アストラガリン   | OGlu           | OH             | H              | OH               | H              | H              | H                | OH               | H              | H               | 2重結合 |
|        | ケルシトリン    | ORha           | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 2重結合 |
|        | ルチン       | ORut           | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 2重結合 |
|        | ミリシトリン    | ORha           | OH             | H              | OH               | H              | H              | OH               | OH               | OH             | H               | 2重結合 |
| フラバノン  | フラバノン     | H              | H              | H              | H                | H              | H              | H                | H                | H              | H               | 単結合  |
|        | ナリンゲニン    | H              | OH             | H              | OH               | H              | H              | H                | OH               | H              | H               | 単結合  |
|        | サクラネチン    | H              | OH             | H              | OCH <sub>3</sub> | H              | H              | H                | OH               | H              | H               | 単結合  |
|        | ヘスペレチン    | H              | OH             | H              | OH               | H              | H              | OH               | OCH <sub>3</sub> | H              | H               | 単結合  |
|        | エリオジクテオール | H              | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 単結合  |
|        | サクラニン     | H              | OGlu           | H              | OCH <sub>3</sub> | H              | H              | H                | OH               | H              | H               | 単結合  |
|        | ナリンジン     | H              | OH             | H              | ORha             | H              | H              | H                | OH               | H              | H               | 単結合  |
|        | ヘスペリジン    | H              | OH             | H              | ORut             | H              | H              | OH               | OCH <sub>3</sub> | H              | H               | 単結合  |
| フラバノール | フスチン      | OH             | H              | H              | OH               | H              | H              | OH               | OH               | H              | H               | 単結合  |
|        | タキシボリン    | OH             | OH             | H              | OH               | H              | H              | OH               | OH               | H              | H               | 単結合  |

[0012] The matter which has the basic structure of a general formula 2 is matter called anthocyanidins, and the compound shown in Table 2 is used in this invention, for example. In addition, the glucose by which a hydrogen atom and OH were \*\*\*\*\* (ed) for H among Table 2, and a methoxy group and OGlu were \*\*\*\*\* (ed) for a hydroxy group and OCH3 is shown.

[0013]



[Formula 7] General formula 2 :

(R1 thru/or R10 show the substituent of arbitration among a formula.)

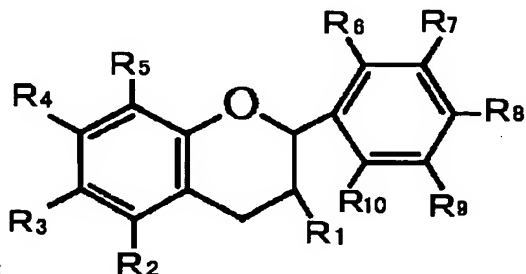
[0014]

[Table 2]

| 名 称           | R1   | R2 | R3 | R4 | R5 | R6 | R7   | R8 | R9   | R10 |
|---------------|------|----|----|----|----|----|------|----|------|-----|
| シアニジン         | OH   | OH | H  | OH | H  | H  | OH   | OH | H    | H   |
| ペラルゴニン        | OH   | OH | H  | OH | H  | H  | H    | OH | H    | H   |
| デルフィニン        | OH   | OH | H  | OH | H  | H  | OH   | OH | OH   | H   |
| ペオニン          | OH   | OH | H  | OH | H  | H  | H    | OH | OCH3 | H   |
| マルビジン         | OH   | OH | H  | OH | H  | H  | OCH3 | OH | OCH3 | H   |
| マルビジン-3-グルコシド | OGLU | OH | H  | OH | H  | H  | OCH3 | OH | OCH3 | H   |

[0015] The matter which has the basic structure of a general formula 3 is matter called flavanols, and the compound shown in Table 3 is used in this invention, for example. In addition, H shows a hydrogen atom among Table 3, and OH shows a hydroxy group.

[0016]



[Formula 8] General formula 3 :

(R1 thru/or R10 show the substituent of arbitration among a formula.)

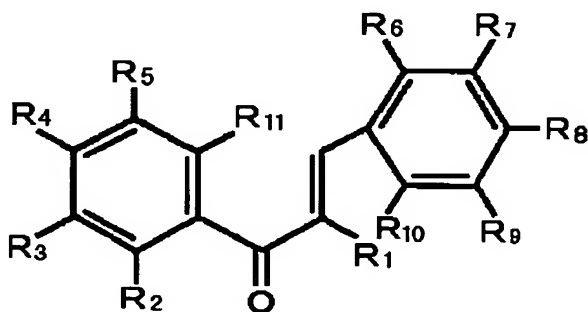
[0017]

[Table 3]

| 名 称      | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
|----------|----|----|----|----|----|----|----|----|----|-----|
| カテキン     | OH | OH | H  | OH | H  | H  | H  | OH | OH | H   |
| エピカテキン   | OH | OH | H  | OH | H  | H  | OH | OH | H  | H   |
| エピガロカテキン | OH | OH | H  | OH | H  | H  | OH | OH | OH | H   |

[0018] The matter which has the basic structure of a general formula 4 is matter called chalcones, and the compound shown in Table 4 is used in this invention, for example. In addition, H shows a hydrogen atom among Table 4, and OH shows a hydroxy group.

[0019]



[Formula 9] General formula 4 :

(R1 thru/or R11 show the substituent of arbitration among a formula.)

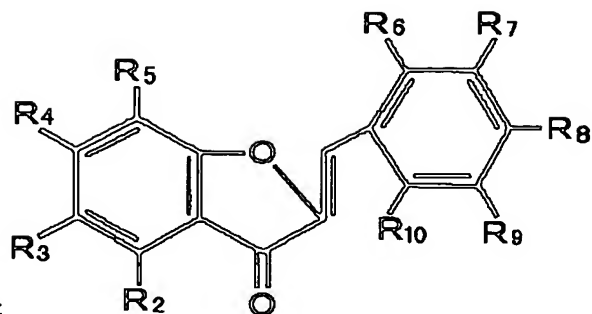
[0020]

[Table 4]

| 名 称   | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 |
|-------|----|----|----|----|----|----|----|----|----|-----|-----|
| カルコン  | H  | H  | H  | H  | H  | H  | H  | H  | H  | H   | H   |
| フロレチン | H  | OH | H  | OH | H  | H  | H  | OH | H  | H   | OH  |

[0021] The matter which has the basic structure of a general formula 5 is matter called Orlon, and the compound shown in Table 5 is used in this invention, for example. In addition, among Table 5, in H, a hydrogen atom and OH show a hydroxy group and OCH3 shows a methoxy group.

[0022]



[Formula 10] General formula 5 :

(R2 thru/or R10 show the substituent of arbitration among a formula.)

[0023]

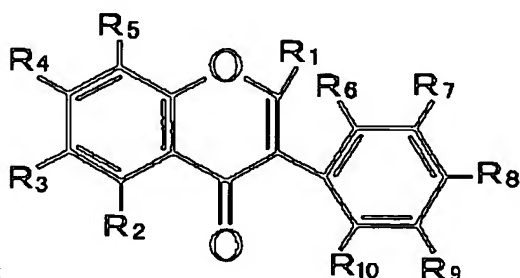
[Table 5]

| 名 称    | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub> | R <sub>5</sub>   | R <sub>6</sub> | R <sub>7</sub> | R <sub>8</sub> | R <sub>9</sub> | R <sub>10</sub> |
|--------|----------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|-----------------|
| スルフレチン | H              | H              | OH             | H                | H              | OH             | OH             | H              | H               |
| レプトシジン | H              | H              | OH             | OCH <sub>3</sub> | H              | OH             | OH             | H              | H               |

[0024] As for the general formula 1 thru/or the matter of 5 used for this invention, i.e., flavones, flavonols, flavanones, FURABANO Norians, anthocyanidins, flavanols, chalcones and Orlon, or the precursor of those, the thing of the gestalt of an aglycon or a glycoside is usually used. Moreover, what is necessary is to be the matter which changes to the matter which has the basic structure which structure changes and is expressed with a general formula 1 thru/or 5 by the metabolic turnover in the living body after intake, and the so-called derivative, and just to be able to reinforce the calcium of a \*\* calcium organization, even if it does not have the basic structure expressed with a general formula 1 thru/or 5 even if it is the derivative which exists in nature, such as those polymers, and. The pro anthocyanidin which is the precursor of a kind of cyanidin of anthocyanidins, the pro anthocyanidin polymer (tannin of a persimmon) which is the polymer of a pro anthocyanidin are one of such things. They are not asked, but the origin and purity are refined further, and if needed, a proper solvent can extract the vegetable ingredient contained comparatively so much, and the obtained extract can be used as it is, or they can use [ a general formula 1 thru/or the matter of 5, or its derivative can raise purity, and ] it. Moreover, it may be compounded artificially, and may be obtained and a commercial item can also be used suitably. Moreover, using if needed as a derivative which was made to combine derivatization of methylation, ethylation, methoxy-izing, ethoxylation, sulfation, \*\*\*\*\*, etc. and water soluble polymers, such as a polyethylene glycol, and raised water solubility and/or stability by proper chemical or biochemical technique is also advantageously made to operation. As such an example, there are alpha-glucosyl rutin (trade name "alphaG rutin" Hayashibara Business-affairs Sale), alpha-glucosyl hesperidin (trade name "alphaG hesperidin" Hayashibara Business-affairs Sale), a methylation hesperidin (the Alps pharmaceutical industry sale), alpha-glycosyl quercetine, alpha-glycosyl naringin, etc. When using the matter which is hard to dissolve in water, if needed, within limits which do not spoil the effectiveness of this invention, it can dissolve with solvents, such as dimethyl sulfoxide and ethanol, or also dissolving and using with sugar, such as trehalose, and using in the state of suspension can also be carried out advantageously.

[0025] Moreover, the general formula 1 thru/or the matter of 5 used for this invention has the description that the operation which strengthens a \*\* calcium organization is remarkably reinforced in multiplication, when it uses together with the isoflavone by which the osteoporosis therapy or the prevention operation is known. Isoflavone is the generic names of the matter which has the structure shown in a general formula 6, and the aglycon and/or its glycoside are used as matter blended with the \*\* calcium organization reinforcement of this invention. For example, in this invention, the matter shown in Table 6 is mentioned and it is blended suitable for the \*\* calcium organization reinforcement of this invention. That isoflavone should just be what can reinforce further the calcification operation to the \*\* calcium organization which the general formula 1 thru/or the matter of 5 used for this invention demonstrates, if needed, it cannot ask, but the origin and purity extract the vegetable ingredient contained comparatively so much with a proper solvent, and the obtained extract can be used as it is, or they can raise and use [ they can be refined further, and ] purity. There is soybean isoflavone as this example. Moreover, it may be compounded artificially, and may be obtained and a commercial item can also be used further suitably. When using the matter which is hard to dissolve in water, it can also dissolve dissolving and using within limits which do not spoil the effectiveness of this invention using solvents, such as dimethyl sulfoxide and ethanol, if needed with sugar, such as alpha and alpha-trehalose, and using in the state of suspension can also be carried out advantageously. Moreover, if needed, it embellishes further by proper chemical or biochemical technique, for example, methylation, ethylation, methoxy-izing, ethoxylation, sulfation, \*\*\*\*\*, etc. are processed, and using as a derivative which raised water solubility and/or stability is also advantageously made to operation. There is alpha-glycosyl isoflavone as such an example.

[0026]



[Formula 11] General formula 6 :

(R1 thru/ or R10 show the substituent of arbitration among a formula.)

[0027]

[Table 6]

| 名 称     | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub>                 | R <sub>5</sub> | R <sub>6</sub> | R <sub>7</sub> | R <sub>8</sub> | R <sub>9</sub> | R <sub>10</sub> |
|---------|----------------|----------------|----------------|--------------------------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| イブプロフェン | H              | H              | H              | OC <sub>3</sub> H <sub>7</sub> | H              | H              | H              | H              | H              | H               |
| ゲニステイン  | H              | OH             | H              | OH                             | H              | H              | H              | OH             | H              | H               |
| ダイゼイン   | H              | H              | H              | OH                             | H              | H              | H              | OH             | H              | H               |
| ゲニステン   | H              | OH             | H              | OGlu                           | H              | H              | H              | OH             | H              | H               |
| ダイジン    | H              | H              | H              | OGlu                           | H              | H              | H              | OH             | H              | H               |

[0028] The \*\* calcium organization as used in the field of this invention is an organization where calcium phosphate salts, such as solid-state crystal-like calcium, for example, hydroxyapatite etc., exist comparatively so much, as such an organization, a bone tissue and a dental organization are especially mentioned, and an osseous tissue and the effectiveness which the \*\* calcium organization reinforcement of this invention has in these organizations are demonstrated.

[0029] The general formula 1 whose \*\* calcium organization reinforcement of this invention is an active principle thru/ or 5 matter, Or the need is accepted in addition to a general formula 1 thru/ or the matter of 5, and isoflavone. For example, silicic acid anhydride, synthetic-aluminum-silicate, lactose, alpha, and alpha-trehalose, Excipients, such as alpha, beta-trehalose, gum arabic, corn starch, and crystalline cellulose, Solvents, such as disintegrator, such as lubricant, such as binders, such as a carboxymethyl cellulose and a polyvinyl pyrrolidone, magnesium stearate, and talc, starch, and carboxymethyl-cellulose calcium, water, and ethanol, etc. can be made to contain. Moreover, it can consider as proper gestalten, such as the shape of powder, granulation, a tablet, the condylus, a liquid, and a paste, and a letter of suspension, by these. About alpha and alpha-trehalose, it can especially use advantageously from the ability to use advantageously as osteoporosis prevention and a remedy as indicated by JP,2000-38343,A and JP,2000-198736,A.

[0030] The general formula 1 contained as an active principle of the \*\* calcium organization reinforcement of this invention thru/ or the matter of 5 or a general formula 1 thru/ or the matter of 5, and isoflavone can use either of mixture with the support or the diluent which can be permitted on combination of only an active principle, or an active principle and pharmaceutical preparation as pharmaceutical preparation. The amount of the active principle in pharmaceutical preparation is anhydride conversion, and is 0.001 % of the weight or more desirably 0.0001% of the weight or more. the \*\* calcium organization reinforcement of this invention — taking orally — parenteral — either can be prescribed for the patient. The dose of the \*\* calcium organization reinforcement of this invention Since sufficient amount for the effectiveness of this invention to demonstrate should be prescribed for the patient according to the administration gestalt and the administration candidate the case written as a general standard although it is meaningless to limit a dose where it will be targeted at Homo sapiens if it becomes — usually — 1 time or several times — dividing — prescribing a medicine for the patient — per day — as the amount of active principles — 0.1 – 500 mg/kg weight — it is 0.5 – 200 mg/kg weight preferably.

[0031] The general formula 1 thru/ or the matter of 5 used as an active principle of the \*\* calcium organization reinforcement of this invention or a general formula 1 thru/ or the matter of 5, and isoflavone can already be extremely prescribed for the patient, taken in or used for insurance as a food additive, a cosmetics raw material, a drugs raw material, etc. also as the ingesta which the many are having safety checked and come to blend this from the first as a \*\* calcium organization reinforcement, cosmetics, or drugs.

[0032] It is applicable to a vertebrate at large [ containing the mammals other than the animal which has the osseous tissue which used solid-state-like calcium as the principal component, and a gear-tooth organization as a candidate for application of the \*\* calcium organization reinforcement of this invention, i.e., a man, and a man ].

[0033] The \*\* calcium organization reinforcement of this invention demonstrates effectiveness to the therapy of the disease about gear teeth, such as a weakened alveolar bone accompanying the disease about bones, such as osteoporosis and fracture, a cavity, and gum disease, the promotion of recovery, or those prevention. Furthermore, it is used also for the promotion of fixing at the time of transplantation of prevention of the bone quantity fall by the osteoclasts by the side effect and deashings of drugs, such as a hormone drug with which the therapy of other diseases etc. was medicated, and a cytokine agent, a natural bone, or an artificial bone. Moreover, it can use for preventing various bone quantity falls also in a healthy person. For example, the fall of the bone quantity by the hormone dyschylia in a woman after the delivery [ before delivery ] in a menopause rear stirrup. The fall of the bone quantity by the hormone dyschylia resulting from too much diet, malnutrition, etc., The fall of the bone quantity by the lack of movement by bedridden [ by paralysis, long-term hospitalization, etc. of bedridden / of elderly people / and the upper membrum inferius ], The fall of the bone quantity resulting from the fall of the bone quantity by the weightlessness by space or underwater life or the gravity low, too much exercise training, etc. is recovered, or the effectiveness of preventing a bone quantity fall in advance is demonstrated. Moreover, it is effective also for the osteogenesis promotion at the time of growth of a childhood person, and bone quantity maintenance of elderly people. Furthermore, since a \*\* calcium organization is made to do the deposition of the calcium in plasma, the effectiveness of improving high calcium plasma depending on operation is also expectable. The above effectiveness is advantageously applicable also to the vertebrate at large which is not limited only to people, for example, contains a dog, a cat, a cow, Buta, a horse, a deer, SAI, an elephant, a wild boar, etc.

[0034] The \*\* calcium organization reinforcement of this invention combined with drugs can expect still higher effectiveness by using together the antiosteoporotic drug conventionally known with the effective dose of the \*\* calcium organization reinforcement of this invention if needed. As antiosteoporotic drug, calcitonin pharmaceutical preparation, an estrogen preparation, bis-phosphonate pharmaceutical preparation, vitamin D pharmaceutical preparation, vitamin K preparation, etc. are mentioned, for example. Moreover, in addition, the narcotic, mesmerism painkiller which are usually used for drugs, An anti-anxiety agent, an antiepileptic agent, an alleviation-of-fever painkilling antiphlogistic, a stimulant, a psycho-stimulant, the anti-Parkin Son agent, The agent for moral nerves, the agent for the central nerves, a skeletal muscle relaxant, the agent for



autonomic nerves, antispasmodic, The agent for ophthalmology, the agent for otolaryngology, an antidiabetic, cardiotonic, the agent for arrhythmia, a diuretic, an antihypertensive, Vasoconstrictor, a coronary vasodilator, a teleangiectasia agent, a hyperlipidemia agent, a respiratory accelerator, Expectorant cough suppressant, a bronchodilator, the agent for allergy, an antidiarrheal drug, a medicine for intestinal disorders, an antiulcer drug, A stomachic digestive, antacid, a choleretic drug, pituitary hormone, a salivary gland hormone agent, A thyroid hormone agent, an anti-thyroid hormone agent, a protein assimilation steroid, an adenocorticotrophic hormone agent, Androgens, follicle hormones, a corpus luteal hormone agent, a mixed hormone drug, A urination reproductive organ agent, an anus agent, the sterilization disinfectant for surgery, a wound protective agent, the external preparations for purulent diseases, A painkiller, an antipruritic agent, an astringent, an antiphlogistic, parasitic skin disease external preparations, an emollient, A caustic drug, dentistry and the agent for the oral cavities, a vitamin compound, miniral preparation, a water addition agent, a hemostat, An anticoagulant, the agent for liver disease, an antidote, the agent for habituation poisoning, a gout drug, Enzyme preparations, the agent for diabetes mellitus, an anticancer drug, an antihistamine, an irritation therapy agent, An antibiotic, a chemotherapeutic drug, biologicals, a parasiticide, an antiprotozoan drug, the agent for preparation, Make drugs, such as an X-ray contrast medium and an agent diagnostic, and a pan make easy intake of the \*\* calcium organization reinforcement of this invention. For example, an adjuvant, an extending agent, a diluent, an excipient, a stabilizer, antiseptics, a coloring agent, Blend 1 or plurality, such as an aromatizing agent, suitably, and a use gestalt is embraced. For example, extracts, elixirs, a capsule, a granule, a pill, ophthalmic ointments, Suspension, an emulsion, plaster, suppositories, powder, spirits, a tablet, syrups, infusa, To decoction, injections, tincture, ophthalmic solutions, the trochiscus, an ointment, cataplasms, aromatic waters, liniments, lemonades, fluid extracts, lotions, drinkable preparations, and a pan It can prepare if needed to a nasal drop, a nasal spray agent, the inhalations for lower respiratory tracts, the gradual release agent for ophthalmology, tunica-mucosa-oris patches, clysters, etc.

[0035] The \*\* calcium organization reinforcement of this invention should just make the active principle of the specified quantity contain by approaches, such as mixing, kneading, the dissolution, immersion, spraying, spreading, spraying, and impregnation, in a process until the preparation is completed.

[0036] Moreover, since the general formula thru/or the matter of 5 used for this invention or a general formula 1 thru/or the matter of 5, and isoflavone fully demonstrate the operation effectiveness which it has, i.e., the operation which makes a \*\* calcium organization do the deposition of the calcium, it is desirable a mineral and to make it especially take in similarly to a lime compound, and it is desirable to make the \*\* calcium organization reinforcement of this invention contain. Moreover, blending with the ingesta, cosmetics, or drugs with which this \*\* calcium organization reinforcement was combined further can also be carried out advantageously. As a lime compound, a calcium chloride, calcium glycerophosphate, calcium gluconate, a calcium lactate, a calcium carbonate, calcium hydrogenphosphate, \*\*\*\*\* hydrogen calcium, calcium hopantenate, calcium pantothenate, a calcium fluoride, calcium thioglycollate, a calcium sulfate, a calcium silicate, calcium acetate, calcium L-aspartate, calcium citrate, malic-acid calcium, succinic-acid calcium, etc. are mentioned, and it can use combining these one sort or two sorts or more.

[0037] Furthermore, in order to demonstrate further the operation effectiveness of the \*\* calcium organization reinforcement of this invention, it is desirable to blend minerals other than calcium, for example, a potassium, sodium, magnesium, manganese, Lynn, iron, zinc, etc. with sufficient balance with the aforementioned lime compound. As for especially a magnesium compound, it is desirable to take in to calcium and coincidence, also when preventing high calcium plasma etc., and the loadings to the \*\* calcium organization reinforcement of this invention of a magnesium compound are desirably blended in 0.5 mols thru/or 0.05 mols to one mol of lime compounds to a lime compound below equimolar. As a magnesium compound, a magnesium oxide, a magnesium chloride, a magnesium carbonate, magnesium sulfate, etc. are mentioned, and it can use, combining suitably these one sort or two sorts or more.

[0038] Moreover, also making the \*\* calcium organization reinforcement of this invention contain the matter which has the mineral absorption promotion operation which helps the parenteral absorption and raises the availability to a living body since it is easy to insolubilize a mineral in intestines, therefore absorption efficiency tends to fall can be carried out advantageously. For example, organic acids, such as oligosaccharides, such as protein [ , such as a casein phospho peptide, ] or oligopeptide, isomalt oligosaccharide, fructo oligosaccharide, xylo oligosaccharide, RAKUTO sucrose, galacto-oligosaccharide, alpha, and alpha-trehalose, alpha, beta-trehalose, and a lactose, a lactic acid, an acetic acid, a citric acid, a gluconic acid, and a succinic acid, are mentioned, and it can use advantageously.

[0039] Moreover, in order to demonstrate further the operation effectiveness of the \*\* calcium organization reinforcement of this invention, it is desirable to make the \*\* calcium organization reinforcement of this invention contain vitamins. As vitamins, an active vitamin D, a vitamin K, L-ascorbic acid, or those derivatives are mentioned.

[0040] Hereafter, an experiment explains this invention.

[0041]

[Experiment 1] Since it was important in the increment in bone quantity to make the osteoblast which has a <retrieval of various organic compounds which make alkaline-phosphatase activity index> calcification operation increase numerically, the matter in which before [ a mouse ] osteoblast stock MC3 T3-E1 cell (RCB1126, RIKEN Gene Bank) is made to specialize to osteoblast was searched about the organic compound known as a vegetable component. As the index, by front osteoblast, activity was low and measurement of the alkaline-phosphatase activity in which it is known in the osteoblast in which front osteoblast specialized that activity is high was adopted.

[0042] By the alpha-MEM culture medium pH 6.8 of the presentation which shows osteoblast stock MC3 T3-E1 in Table 7 containing 10mMHEPES(s) and 10(v/v) % fetal calf serum (the Gibco BIARUERU sale) before a <culture of osteoblast before mouse> mouse It cultivated in 37 degrees C and 5% carbon-dioxide-gas ambient atmosphere until it prepared to 5x10<sup>4</sup> cell concentration/ml, it carried out seeding of the 5x10<sup>4</sup> cells per every ml per hole to 24 hole plate (falcon BEKUTON DIKKINSON sale) and the cell adhered to the plate base. The organic compound which dissolved in ethanol after culture-medium removal, i.e., indomethacin, (Funakoshi Co., Ltd. sale) (positive control) Kaempferol (Funakoshi Co., Ltd. sale), a gallic acid (Nakarai Tesuku, Inc. sale), Coffeic acid (the Wako Pure Chem Industries sale), 4-methylumbelliferone (the Wako Pure Chem Industries sale), Caffeine (the Wako Pure Chem Industries sale), beta carotene (sigma company sale), Glycyrrhizin (the Katayama chemical-industry incorporated company sale), menthol (the Wako Pure Chem Industries sale), Theophylline (the Wako Pure Chem

Industries sale), a tocopherol (sigma company sale), It added at a time 1ml (10% (v/v) fetal calf serum and 10mM beta-glycero phosphate are included) of alpha-MEM culture media which contain a vanillin (the Wako Pure Chem Industries sale) by the concentration of 20microM, respectively. In addition, since 0.2% (v/v) ethanol of the last concentration was contained in the culture medium, as a negative control, it considered as the alpha-MEM culture medium which contains ethanol 0.2% (v/v). Continuous culture was carried out for eight days, exchanging for the culture medium which contains each fresh sample every three days, or the culture medium which is not included.

[0043]

[Table 7]

| 成分         | mg/l | 成分             | mg/l  |
|------------|------|----------------|-------|
| L-アラニン     | 25   | L-アスコルビン酸      | 50    |
| L-アルギニン    | 105  | ビオチン           | 0.1   |
| L-アスパラギン   | 25   | コリン            | 1     |
| L-アスパラギン酸  | 30   | シアノコバラミン       | 1.38  |
| L-システイン    | 100  | 葉酸             | 1     |
| L-シスチン     | 24   | イノシトール         | 2     |
| L-グルタミン酸   | 75   | ニコチンアミド        | 1     |
| L-グルタミン    | 292  | パントテン酸         | 1     |
| グリシン       | 50   | ピリドキサル         | 1     |
| L-ヒシチジン    | 31   | リボフラビン         | 0.1   |
| L-イソロイシン   | 52   | チアミン           | 1     |
| L-ロイシン     | 52   | 塩化カルシウム        | 200   |
| L-リジン      | 58   | 塩化カリウム         | 400   |
| L-メチオニン    | 15   | 塩化マグネシウム       | 200   |
| L-フェニルアラニン | 32   | 塩化ナトリウム        | 8,800 |
| L-プロリン     | 40   | 炭酸水素ナトリウム      | 2,000 |
| L-セリン      | 25   | リン酸2水素ナトリウム    | 150   |
| L-スレオニン    | 47.8 | グルコース          | 1,000 |
| L-トリプトファン  | 10   | リボ酸            | 0.2   |
| L-チロシン     | 38   | フェノールスルホンフタレイン | 10    |
| L-バリン      | 48   | ビルビン酸ナトリウム     | 110   |

[0044] Measurement of <measurement of alkaline-phosphatase activity> alkaline-phosphatase activity was performed according to KAJI's and others approach using alkali phosphatase Test Wako (the Wako Pure Chem Industries sale) (ACHIBUZU OBU oral biology (Archives of Oral Biology), the 44th volume, 233, or 241 pages (1999)). That is, after removing a culture medium from the above-mentioned osteoblast before a mouse, and 1ml of 0.25M sucrose solutions washing 3 times, carrying out 0.05ml sequential addition of the 50mM carbonate buffer solution pH 9.8 containing 1mM magnesium chloride and 50mM sucrose and leaving 0.45ml and 25 degree C of 3.4mM p-nitrophenyl phosphoric-acid NINATORIUMU for 5 minutes, the reaction was stopped by adding 1.5ml of 0.6-N sodium hydroxides. The absorbance with a wavelength of 405nm was measured for the amount of p-nitrophenol generated according to an operation of the alkaline phosphatase in the above-mentioned reaction mixture, and the value was made into alkaline phosphatase activity. The relative activity which showed the ratio of the alkaline-phosphatase activity in each sample and the activity of the negative control which added only the culture medium which contains ethanol 0.2% (v/v) in Table 8 was summarized.

[0045]

[Table 8]

| 有機化合物          | 相対アルカリフォスファターゼ活性(%) |
|----------------|---------------------|
| 0.2%(v/v)エタノール | 100                 |
| インドメタシン        | 142                 |
| ケンフェロール        | 162                 |
| 没食子酸           | 79                  |
| カフェ酸           | 97                  |
| 4-メチルウンベリフェロン  | 96                  |
| カフェイン          | 100                 |
| β-カロテン         | 81                  |
| グリチルリチン酸       | 97                  |
| メントール          | 95                  |
| デオフィリン         | 92                  |
| トコフェロール        | 100                 |
| バニリン           | 89                  |

[0046] From the result of Table 8, kaempferol reinforced the alkaline-phosphatase activity of a before [ a mouse ] osteoblast stock (MC3 T3-E1) rather than the indomethacin which is an electropositive object. From this result, since kaempferol reinforced the alkaline-phosphatase activity of the osteoblast before a mouse, it became clear that it had the operation which makes front osteoblast specialize in osteoblast.

[0047]

[Experiment 2] In the <retrieval of various organic compounds which made the calcification operation index> experiment 1, the

differentiation-inducing promotion operation to the osteoblast which the subject sample containing various organic compounds has from front osteoblast was investigated. Next, it investigated whether the calcification operation would actually be reinforced. As an organic compound, indomethacin (Funakoshi Co., Ltd. selling sale) (positive control), kaempferol (Funakoshi Co., Ltd. sale), the hesperetin (Funakoshi Co., Ltd. sale), a gallic acid (Nakarai Tesuku, Inc. sale), and caffeic acid (the Wako Pure Chem Industries sale) were used. It added at a time 1ml (10% (v/v) fetal calf serum and 10mM beta-glycero phosphate are included) of alpha-MEM culture media which contain these by the concentration of 20microM, respectively to mouse MC3 T3-E1 cell strain. In addition, since 0.2% (v/v) ethanol of the last concentration was contained in the culture medium, as a negative control, it considered as the alpha-MEM culture medium which contains ethanol 0.2% (v/v). Continuous culture was carried out for eight days, exchanging for the culture medium which contains each fresh sample every three days, or the culture medium which is not included. According to the conventional method, the alizarin-red-S staining technique was performed for these, and the amount of calcification was measured. That is, after removing a culture medium from a culture plate, it washed twice by 1ml of Dulbecco-phosphate buffered saline, 1ml of 50(v/v) % ethanol water solutions was added, and it was left at the room temperature for 10 minutes. After removing added 50(v/v) % ethanol water solution, 1ml of distilled water was added and it was again left at the room temperature for 10 minutes. After having added 1ml of alizarin-red-S (the Wako Pure Chem Industries sale) solutions 1% (w/v) after removing added distilled water, and leaving it for 10 minutes, the alizarin-red-S solution was removed and 1ml of distilled water washed 3 times. Since the calcium which carried out deposition was dyed red in the culture plate base by the above-mentioned processing, the macro-scopical judging of the amount of calcification was carried out by the degree of reddening. The criterion made +++ dyeing comparable as + and positive control (indomethacin) for dyeing comparable as a negative control (only culture medium which contains ethanol 0.2% (v/v)), and made ++ the degree of dyeing of about 50 percent of dyeing of positive control. A result is shown in Table 9.

[0048]

[Table 9]

| 有機化合物                  | カルシウム沈着量 |
|------------------------|----------|
| 0.2%(v/v) エタノール (陰性対照) | +        |
| インドメタシン                | +++      |
| ケンフェロール                | +++      |
| ヘスペレチン                 | +++      |
| 没食子酸                   | +        |
| カフェ酸                   | +        |

[0049] From the result of Table 9, it was judged with kaempferol and the hesperetin being the amounts of calcification almost equivalent to the indomethacin adopted as positive control. From this result, it became clear that kaempferol and the hesperetin had the operation which reinforces the calcification of front osteoblast.

[0050] It became clear that kaempferol has the operation which makes the alkaline phosphatase activity of front osteoblast increase, i.e., the operation which makes front osteoblast specialize in osteoblast, from the result of experiments 1 and 2, and that kaempferol and the hesperetin had the operation which reinforces the calcification.

[0051]

[Experiment 3] It investigated whether it would have effectiveness with the same said of the compound belonging to <comparison in various flavonoid> kaempferol, or other flavonoid other than the hesperetin. A flavone (the Kanto chemistry incorporated company sale), apigenin (Funakoshi Co., Ltd. sale), Flavonol (Tokyo formation industrial incorporated company sale), quercetine (the Kanto chemistry incorporated company sale), Kaempferol (Funakoshi Co., Ltd. sale), rutin (the Kanto chemistry incorporated company sale), A flavanone (the Kanto chemistry incorporated company sale), a naringenin (Aldrich sale), The hesperetin (Funakoshi Co., Ltd. sale), a hesperidin (the Kanto chemistry incorporated company sale), A chalcone (Merck Co. sale), phloretin (sigma company sale), a catechin (sigma company sale), Ipriflavone (DAITO, Inc. sale), taxi HORIN (Funakoshi Co., Ltd. sale), SURUFURECHIN (Funakoshi Co., Ltd. sale), and cyanidin (Funakoshi Co., Ltd. sale) were adopted as a subject sample. Alkaline phosphatase activity was measured by the approach of experiment 1. On the other hand, measurement of the amount of calcification was performed according to the conventional method using calcium C Test Wako (Wako Pure Chem make). That is, after removing a culture medium from a cultured cell and washing 3 times by 1ml of Dulbecco-phosphate buffered saline, in order to dissolve the calcium which carried out deposition, 0.5ml of 2-N hydrochloric acids was added. 5microl was extracted from this solution, and 0.63mM o-cresol phthalein-complexon (calcium ion and chelate complex are formed.) 0.05ml which contains 0.5ml and 69mM eight quinolinol for the 0.88M monoethanolamine buffer solution (pH11) was added and mixed. As compared with the value which measured the absorbance with a wavelength of 570nm for this, and measured the standard calcium solution on this wavelength, the amount of calcium around plate 1 hole was computed. The evaluation approach was performed by comparing in the negative control alkaline-phosphatase activity which added only the culture medium which contains ethanol 0.2% (v/v), respectively about the alkaline-phosphatase activity and the amount of calcification in each sample, the relative activity over the amount of calcification, and a relative amount. A result is shown in Table 10.

[0052]

[Table 10]

|                | 相対アルカリフォ<br>スファターゼ活<br>性(%) | 相対カルシウム<br>沈着量(%) |
|----------------|-----------------------------|-------------------|
| 0.2%(v/v)エタノール | 100                         | 100               |
| フラボン           | 103                         | 158               |
| アピゲニン          | 128                         | 247               |
| フラボノール         | 139                         | 295               |
| ケルセチン          | 120                         | 118               |
| ケンフェロール        | 162                         | 305               |
| ルチン            | 109                         | 74                |
| フラバノン          | 109                         | 311               |
| ナリンゲニン         | 110                         | 271               |
| ヘスペレチン         | 116                         | 289               |
| ヘスペリジン         | 107                         | 118               |
| カルコン           | 105                         | 104               |
| フロレチン          | 109                         | 238               |
| カテキン           | 118                         | 247               |
| イプリフラボン        | 114                         | 263               |
| タキシホリン         | 98                          | 234               |
| スルフレチン         | 126                         | 157               |
| シアニジン          | 115                         | 216               |

[0053] From the result of Table 10, the increment in the amount of calcification was accepted in the flavonoid made into the active principle of this invention. It became clear that the operation effectiveness which front osteoblast is made to specialize in osteoblast and reinforces the deposition of calcium from this result was a thing common to flavones, flavonols, flavanones, FURABANO Norians, anthocyanidins, flavanols, chalcones, or Orlon.

[0054]

[Experiment 4] The reinforcement of the operation which makes osteoblast carry out differentiation inducing of the front osteoblast was measured with <comparison of kaempferol [ in alkaline phosphatase activity enhancement ], hesperetin, or operation effectiveness of ipriflavone> kaempferol, or the hesperetin, using the ipriflavone already known as a remedy of osteoporosis as a subject sample. The culture days in the inside of the culture medium containing each subject sample were performed on three days, six days, and the 9th according to the approach of experiment 1 except having set subject sample concentration in a culture medium to 5, 10, and 20microM. The ethanol concentration of a culture medium becomes 0.2% (v/v) 0.1% (v/v) 0.05% (v/v) at the time of 20microM at the time of 10microM, when each \*\*\*\* sample concentration is 5microM. What is the same culture medium as these ethanol concentration, and does not contain a subject sample was prepared as contrast, and the relative activity over the activity of contrast was searched for. A result is shown in drawing 1.

[0055] In the case of concentration [ 20micro ] M, from the result of drawing 1, the subject sample was a little overdue by kaempferol, enhancement of remarkable alkaline-phosphatase activity was accepted in six days of culture from it, and although activity was a little inferior in the subject sample by the hesperetin compared with ipriflavone, enhancement of remarkable alkaline-phosphatase activity was accepted in three days of culture. From this result, it became clear that the effectiveness of reinforcing the alkaline phosphatase activity of a before [ a mouse ] osteoblast stock (MC3 T3-E1) of kaempferol and the hesperetin was equal to the effectiveness of the ipriflavone as an osteoporosis agent.

[0056]

[Experiment 5] The amount potentiation \*\*\*\*\* of <comparison of operation effectiveness of kaempferol [ in calcification enhancement ] and ipriflavone> calcification and kaempferol were compared with ipriflavone. The addition approach of a subject sample and a subject sample was performed like the experiment 4. Measurement of the amount of calcification was performed according to the conventional method using calcium C Test Wako (Wako Pure Chem make). That is, after removing a culture medium from a cultured cell and washing 3 times by 1ml of Dulbecco-phosphate buffered saline, in order to dissolve the calcium which carried out deposition, 0.5ml of 2-N hydrochloric acids was added. 5microl was extracted from this solution, and 0.63mM o-cresol phthalein-complexon (calcium ion and chelate complex are formed.) 0.05ml which contains 0.5ml and 69mM eight quinolinol for the 0.88M monoethanolamine buffer solution (pH11) was added and mixed. As compared with the value which measured the absorbance with a wavelength of 570nm for this, and measured the standard calcium solution on this wavelength, the amount of calcium around plate 1 hole was computed. What kaempferol and ipriflavone compared is shown in drawing 2. In addition, what was processed only by the ethanol of contrast is shown in drawing.

[0057] From the result of drawing 2, kaempferol showed the outstanding calcification potentiation rather than ipriflavone.

[0058] From experiments 4 and 5, kaempferol and the hesperetin having the rise of the same alkaline-phosphatase activity as the ipriflavone known as a conventional anti-osteoporosis medicine and calcification potentiation to the osteoblast before a mouse, and having the operation which strengthens a \*\* calcium organization was shown.

[0059]

[Experiment 6] The potentiation of the calcification by concomitant use with the ipriflavone which is isoflavone was investigated using <combined effect of kaempferol [ in calcification enhancement ], ipriflavone, or hesperetin and ipriflavone> kaempferol, or the hesperetin. According to the experiment 1, the measuring method of calcium was performed according to the experiment 3 and the experiment 5 except the preparation approach of a sample and the culture approach of a cell having made five days the culture days in the inside of the culture medium containing each subject sample. In addition, ethanol concentration contained in the culture medium containing each \*\*\*\* sample was made the same. As a subject sample, kaempferol 5microM, 10microM, 20microM independence, Ipriflavone, respectively with ipriflavone 5microM, 10microM, 20microM independence, or kaempferol

5microM, Ipriflavone was shown in drawing 3 with hesperetin 5microM, 10microM, 20microM independence, ipriflavone 5microM, 10microM, 20microM independence, or the hesperetin, and 5microM, 10microM, and the thing of which 20microM concomitant use was done were shown for 10microM and the thing of which 20microM concomitant use was done in drawing 4, respectively. [0060] From the result of drawing 3 and drawing 4, to front osteoblast, when kaempferol and the hesperetin used together with ipriflavone, the calcification was reinforced more remarkably than the case of being independent, and the synergistic effect by concomitant use was accepted.

[0061]

[Experiment 7] Six <calcification enhancing effect in rat femur and tibia> 3-weeks old feminity Wistar system rats were used as one group. Each subject sample (Funakoshi Co., Ltd. sale), i.e., the hesperetin, quercetine (Tokyo, Inc. formation sale), alpha-glycosyl hesperidin (the trade name "alphaG hesperidin P" Hayashibara business-affairs incorporated company sale), alpha-glycosyl rutin (the trade name "alphaG rutin P" Hayashibara business-affairs incorporated company sale), The one ipriflavone (DAITO, Inc. sale) (positive control) weight each section was blended with the high sucrose addition feed (each numeric value in Table 11 shows weight %) of the rate of a compounding ratio shown in Table 11, and the rat was given by making it into food. The rat which gave the food which added the sucrose 1 weight section instead of the subject sample was considered as contrast. Water was considered as free intake. The rat was slaughtered after eight weeks of test initiation, and the femur and the tibia were extracted. The femur and tibia which were extracted measured dry weight with the upper pan electronic balance for high precision analysis (trade name "HA180M/12QM" EANDO, Inc. dee sale), after making it dry at 100 degrees C for about 6 hours. next, a femur and a tibia — a crucible — putting in — ashing — after processing for 6 hours and fully ashing 950 degrees C at a furnace, it was made to dissolve in hydrochloric-acid liquid, and the quantum of the amount of calcium was carried out with the atomic absorption spectro-photometer by making this into a sample solution. The amount of calcium computed the amount of calcium per desiccation bone, and measured it with contrast. A result is shown in Table 12.

[0062]

[Table 11]

| 飼料成分<br>(重量%) | 試 験 群      |       |                |                       |             | なし<br>(対照群) |
|---------------|------------|-------|----------------|-----------------------|-------------|-------------|
|               | ヘスペレ<br>テン | ケルセチン | α-グリコ<br>シルルチン | α-グリコ<br>シルヘスペ<br>リジン | イプリ<br>フラボン |             |
| フラボノイド類(試料)   | 0.5        | 0.5   | 1.25           | 1.25                  | 0.5         | 0           |
| コーンスターチ       | 14.5       | 14.5  | 13.75          | 13.75                 | 14.5        | 15          |
| スクロース         | 50         |       |                |                       |             |             |
| カゼイン          | 20         |       |                |                       |             |             |
| セルロースパウダー     | 5          |       |                |                       |             |             |
| コーンオイル        | 5          |       |                |                       |             |             |
| ミネラル類         | 3.5        |       |                |                       |             |             |
| ビタミン類         | 1.2        |       |                |                       |             |             |
| メチオニン         | 0.3        |       |                |                       |             |             |

[0063]

[Table 12]

| 試 料               |     | 乾燥骨重量<br>(g) | 乾燥骨一本当<br>りのカルシウ<br>ム重量 (mg) | 備 考  |
|-------------------|-----|--------------|------------------------------|------|
| ヘスペレチン            | 大腿骨 | 0.463        | 125.1                        | 本発明  |
|                   | 脛 骨 | 0.357        | 109.0                        |      |
| ケルセチン             | 大腿骨 | 0.456        | 120.3                        | 本発明  |
|                   | 脛 骨 | 0.352        | 108.7                        |      |
| α-グリコシル<br>ルチン    | 大腿骨 | 0.439        | 119.9                        | 本発明  |
|                   | 脛 骨 | 0.343        | 107.7                        |      |
| α-グリコシル<br>ヘスペリジン | 大腿骨 | 0.454        | 126.9                        | 本発明  |
|                   | 脛 骨 | 0.349        | 109.5                        |      |
| イプリフラボン           | 大腿骨 | 0.428        | 120.0                        | 陽性対照 |
|                   | 脛 骨 | 0.338        | 108.0                        |      |
| な し               | 大腿骨 | 0.427        | 116.4                        | 陰性対照 |
|                   | 脛 骨 | 0.331        | 105.9                        |      |

[0064] From the result of Table 12, in any [ of each subject sample ] case, the amount of calcification of a femur and a tibia reinforced compared with the negative control, and was the ipriflavone of positive control, an EQC, or more than an EQC. Moreover, the increment in the amount of calcification was accepted also in glycosides, such as a hesperidin, alpha-glycosyl hesperidin, and alpha-glycosyl rutin. In addition, significant potentiation was not accepted when in vitro alkaline-phosphatase activity and calcification potentiation were investigated using these glycosides. Therefore, in order to demonstrate such effectiveness, each flavonoid needs to take the gestalt of an aglycon, the part of sugar is decomposed by the inside of an intestinal tract, or the enzyme in the living body in in vivo administration, and becoming an aglycon and acting is guessed.

[0065]

[Experiment 8] The <acute toxicity test> 4-weeks old Wistar system feminity rat was used one groups [ five ]. Each organic

compound used in the experiment 3 as a subject sample, i.e., a flavone, (the Kanto chemistry incorporated company sale) Apigenin (Funakoshi Co., Ltd. sale), flavonol (Tokyo formation industrial incorporated company sale), Quercetine (the Kanto chemistry incorporated company sale), kaempferol (Funakoshi Co., Ltd. sale), Rutin (the Kanto chemistry incorporated company sale), a flavanone (the Kanto chemistry incorporated company sale), A naringenin (Aldrich sale), the hesperetin (Funakoshi Co., Ltd. sale), A hesperidin (the Kanto chemistry incorporated company sale), a chalcone (Merck Co. sale), Phloretin (sigma company sale), a catechin (sigma company sale), ipriflavone (DAITO, Inc. sale), The one weight each section is dissolved or suspended for taxi HORIN (Funakoshi Co., Ltd. sale), SURUFURECHIN (Funakoshi Co., Ltd. sale), or cyanidin (Funakoshi Co., Ltd. sale) in the 5 (w/v) % gum arabic solution 25 weight section. The stomach tube was used for the rat which abstained from food for 18 hours, and forcible internal use was carried out by the dosage of 10 ml/kg rat weight. henceforth, a rat — constant temperature — it bred on condition that constant humidity, and food and water were given freely. The general symptom and the life-and-death condition were observed for 14 days, measuring the weight of an each object having used the administration day as zero day. [0066] After breeding for two weeks, there was no example of death in every subject sample, and there was no loss weight, the appearance is good and the thing remarkable as a general symptom was not accepted. Therefore, the general formula 1 thru/or the matter of 5 used for the \*\* calcium organization reinforcement of this invention as an active principle is judged to be what has very high safety.

[0067] Hereafter, the example of this invention is explained.

[0068]

[Example 1] After dissolving the <liquids-and-solutions> alpha-glycosyl rutin (trade name "alphaG rutin P" Hayashibara Business-affairs Sale) 1 weight section and the quercetine (Funakoshi Co., Ltd. sale) 0.1 weight section in the ethanol 50 weight section, furthermore, water 5,000 weight section, alpha, and alpha-trehalose (a trade name "TOREHA" —) The Hayashibara, Inc. business-affairs selling 50 weight section, the calcium-lactate 3 weight section, the magnesium chloride 1.5 weight section, and the casein phospho peptide 10 weight section are added, the mixed dissolution was carried out, and the liquefied \*\* calcium organization reinforcement was obtained.

[0069] Since there is effectiveness which maintains and reinforces a \*\* calcium organization, this article with which the calcium which can take easily and is excellent in absorption efficiency is blended can be used in favor of a therapy and prevention, or promotion of recovery of osteoporosis, fracture, etc.

[0070]

[Example 2] a <liquids-and-solutions> alpha-glycosyl hesperidin (a trade name "the alphaG hesperidin PA" —) After dissolving the Hayashibara, Inc. business-affairs selling 1 weight section and the hesperetin (Funakoshi Co., Ltd. sale) 0.1 weight section in the ethanol 50 weight section, furthermore, water 5,000 weight section, alpha, and alpha-trehalose (a trade name "TOREHA" —) The Hayashibara, Inc. business-affairs selling 20 weight section, the calcium chloride 5 weight section, the magnesium chloride 2.5 weight section, and the RAKUTO sucrose 10 weight section are added, the mixed dissolution was carried out, and the liquefied \*\* calcium organization reinforcement was obtained.

[0071] Since there is effectiveness which maintains and reinforces a \*\* calcium organization, this article with which the calcium which can take easily and is excellent in absorption efficiency is blended can be used in favor of a therapy and prevention, or promotion of recovery of osteoporosis, fracture, etc.

[0072]

[Example 3] The <liquids-and-solutions> sodium chloride 6 weight section, the potassium chloride 0.3 weight section, the calcium chloride 0.2 weight section, The powdered alpha-glucosyl quercetine 1 weight section obtained by the approach of the example A-2 of the sodium lactate 3.1 weight section, alpha, and beta-trehalose 45 weight section and a JP,5-32690,A official report is dissolved in the water 1,000 weight section. According to the conventional method, purification filtration was carried out, it considered as the pyrogen free-lancer, 25ml ampul which sterilized this solution was filled up, and the \*\* calcium organization reinforcement for injection was obtained.

[0073] Since there is effectiveness which maintains and reinforces a calcium organization as injections, this article can be used in favor of a therapy and prevention, or promotion of recovery of osteoporosis, fracture, etc.

[0074]

[Example 4] After mixing the <powder-material> alpha-glycosyl rutin (trade name "alphaG rutin H" Hayashibara Business-affairs Sale) 1 weight section, soybean isoflavone (FUJI OIL Co., Ltd. sale) 5 weight section, alpha, and alpha-trehalose powder (trade name "TOREHA" Hayashibara Business-affairs Sale) 1000 weight section, the calcium-lactate 1 weight section, and the magnesium sulfate 0.5 weight section with homogeneity, it dried and the powder-like \*\* calcium organization reinforcement was manufactured.

[0075] Since stable this article is easily dissolvable and also contains calcium and magnesium to water, it has the outstanding effectiveness which strengthens a \*\* calcium organization. It is useful as health food, such as a health supplement and health functional food, as the therapy of osteoporosis, fracture, etc., and a \*\* calcium organization reinforcement for preventing.

[0076]

[Example 5] a <powder-material> alpha-glycosyl hesperidin (a trade name "the alphaG hesperidin H" —) Hayashibara, Inc. business-affairs selling 1 weight section, alpha, and alpha-trehalose powder (a trade name "TOREHA" —) The Hayashibara, Inc. business-affairs selling 1000 weight section, the calcium-lactate 1 weight section, After mixing the magnesium sulfate 0.5 weight section and the L-ascorbic acid-2-glucoside (trade name "AA2G" Hayashibara Business-affairs Sale) 3 weight section with homogeneity, it dried and the powder-like \*\* calcium organization reinforcement was manufactured.

[0077] Since stable this article is easily dissolvable and also contains calcium and magnesium to water, it has the outstanding effectiveness which strengthens a \*\* calcium organization. It is useful as health food, such as a health supplement and health functional food, as the therapy of osteoporosis, fracture, etc., and a \*\* calcium organization reinforcement for preventing.

[0078]

[Example 6] a <trochiscus> alpha-glycosyl hesperidin (a trade name "the alphaG hesperidin PA" —) The Hayashibara, Inc. business-affairs selling 1 weight section and the hesperetin (Funakoshi Co., Ltd. sale) 0.1 weight section are dissolved in the ethanol 1 weight section, calcium-lactate 2 weight section, gum arabic 10 weight section, alpha, and alpha-trehalose powder (a trade name "TOREHA" —) The Hayashibara, Inc. business-affairs selling 10 weight section, the Scralose (San-Ei Gen F.F.I., Inc.



sale) 5 weight section, and the water 3 weight section were often mixed, it fabricated with the conventional method, and the trochiscus was obtained.

[0079] Stable this article is useful as trochiscus which maintains and reinforces the amount of calcium of an alveolar bone and a gear tooth.

[0080]

[Example 7] The <trochiscus> alpha-glycosyl rutin (trade name "alphaG rutin P" Hayashibara Business-affairs Sale) 1 weight section, And the quercetine (Funakoshi Co., Ltd. sale) 0.1 weight section is dissolved in the ethanol 1 weight section. The calcium-lactate 2 weight section, gum arabic 10 weight section, alpha, and alpha-trehalose powder (trade name "TOREHA" Hayashibara Business-affairs Sale) 10 weight section, the sugar transition stevia 2 weight section, and the water 3 weight section were often mixed to this, it fabricated with the conventional method, and the trochiscus was obtained.

[0081] Stable this article is useful as trochiscus which maintains and reinforces the amount of calcium of an alveolar bone and a gear tooth.

[0082]

[Example 8] The <health supplement> alpha and alpha-trehalose powder (trade name "TOREHA" Hayashibara Business-affairs Sale) 52 weight section, the corn-starch 40 weight section and alpha-glycosyl rutin (a trade name "alphaG rutin P" —) the Hayashibara, Inc. business-affairs selling 0.5 weight section and alpha-glycosyl hesperidin (a trade name "the alphaG hesperidin PA" —) the Hayashibara, Inc. business-affairs selling 0.5 weight section and a L-ascorbic acid-2-glucoside (a trade name "AA2G" —) After kneading and carrying out fluid bed granulation, having mixed the Hayashibara, Inc. business-affairs selling calcium chloride 0.1 weight section and the crystalline cellulose 2.5 weight section, and carrying out spraying dropping of the water of optimum dose according to a conventional method, the particle size regulation was ground and carried out and the fine particles for making tablets were obtained. After mixing the sucrose-fatty-acid-ester 2 weight section to homogeneity as an abundant agent at this, it tableted with the tableting machine equipped with a pestle with a diameter of 11mm, and the tablet (about 300mg / lock) was obtained.

[0083] This article which was easy to take in and was excellent in the collapsibility in an alimentary canal is useful as health food, such as a health supplement which maintains and reinforces bone quantity, and health functional food.

[0084]

[Example 9] The <drinkable-preparations> alpha-glycosyl rutin (trade name "alphaG rutin P" Hayashibara Business-affairs Sale) 1 weight section, The alpha-glycosyl naringin 1 weight section manufactured according to the approach indicated by the JP,4-13691,A example A-2, grapefruit fruit-juice 20 weight section, alpha, and alpha-trehalose powder (a trade name "TOREHA" —) the Hayashibara, Inc. business-affairs selling 2 weight section, the citric-acid 2 weight section, and a L-ascorbic acid-2-glucoside (a trade name "AA2G" —) It sealed and drinkable preparations were obtained, after mixing the Hayashibara, Inc. business-affairs selling 1 weight section, the isomerized sugar 5 weight section, the seawater 6 weight section, the calcium chloride 2 weight section, and the water 160 weight section and filling up a carboy with 100ml at a time.

[0085] On flavor fitness, since this article has the operation which strengthens a \*\* calcium organization, it is useful as the therapy of osteoporosis, fracture, etc., and drinkable preparations for preventing.

[0086]

[Example 10] It sealed and drinkable preparations were obtained, after mixing the <drinkable-preparations> alpha-glycosyl hesperidin (trade name "alphaG hesperidin PA" Hayashibara Business-affairs Sale) 1 weight section, apple fruit-juice 50 weight section, isomerized sugar 5 weight section, L-ascorbic acid 2 weight section, alpha, and alpha-trehalose (trade name "TOREHA" Hayashibara Business-affairs Sale) 3 weight section, the monosodium L-aspartate 2 weight section, and the water 35 weight section and filling up a carboy with 100ml at a time.

[0087] On flavor fitness, since this article has the operation which strengthens a \*\* calcium organization, it is useful as the therapy of osteoporosis, fracture, etc., and drinkable preparations for preventing.

[0088]

[Example 11] A <paste agent> conventional method is followed. The calcium diphosphate 45 weight section, The pullulan 3 weight section, the sodium-lauryl-sulfate 1.5 weight section, the glycerol 20 weight section, The polyoxyethylenesorbitanlaurate 0.5 weight section, the sorbitol 10 weight section, the maltitol 7 weight section and the purified water 12 weight section — alpha-glycosyl hesperidin (a trade name "the alphaG hesperidin PA" —) The ethanol solution 1 weight section which contains the 0.4 weight section and the hesperetin (Funakoshi Co., Ltd. sale) 0.1 weight section for Hayashibara Business-affairs Sale was blended, and the paste agent was obtained.

[0089] Stable this article is useful as tooth paste which maintains and reinforces the amount of calcium of an alveolar bone and a gear tooth.

[0090]

[Example 12] A <paste agent> conventional method is followed. The calcium diphosphate 45 weight section, The pullulan 3 weight section, the sodium-lauryl-sulfate 1.5 weight section, the glycerol 20 weight section, The polyoxyethylenesorbitanlaurate 0.5 weight section, the sorbitol 10 weight section, The ethanol solution 1 weight section which contains the 0.4 weight section and the quercetine (Funakoshi Co., Ltd. sale) 0.1 weight section for alpha-glycosyl rutin (trade name "alphaG rutin P" Hayashibara Business-affairs Sale) was blended with the maltitol 7 weight section and the purified water 12 weight section, and tooth paste was obtained.

[0091] Stable this article is useful as tooth paste which maintains and reinforces the amount of calcium of an alveolar bone and a gear tooth.

[0092]

[Effect of the Invention] The \*\* calcium organization reinforcement containing a general formula 1 thru/or the matter of 5 Since strong calcification activity is shown and the operation is further reinforced remarkably in multiplication by adding isoflavone in front osteoblast or osteoblast It has an osteogenesis facilitatory effect and the increment effectiveness in bone quantity in a bone growth list, and can take in easily [ there is no sense of incongruity also as ingesta which it makes health food, such as a health supplement and health functional food, contain, and it not only can take in, but eats daily and ]. Especially the calcium organization reinforcement of this invention is useful also to the therapy and prevention of osteoporosis while it prevents elderly

people's bone and a weakened gear tooth. Furthermore, also in the younger age group, although delay of the bone growth and osteogenesis which are produced by the modulation of eating habits is prevented, high effectiveness is done so.

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[Translation done.]



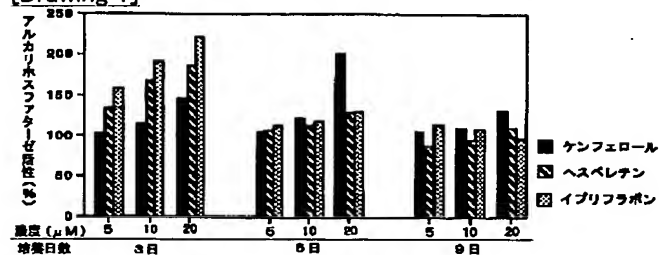
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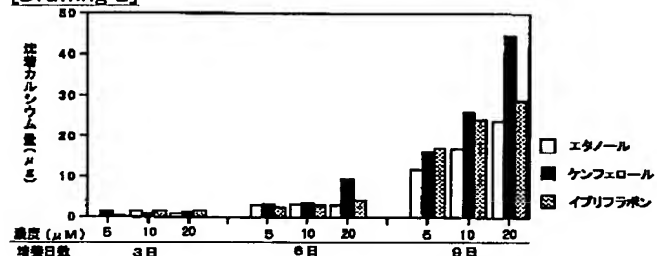
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DRAWINGS

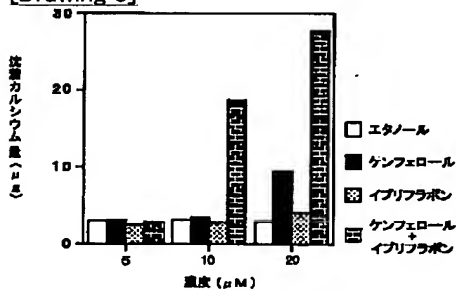
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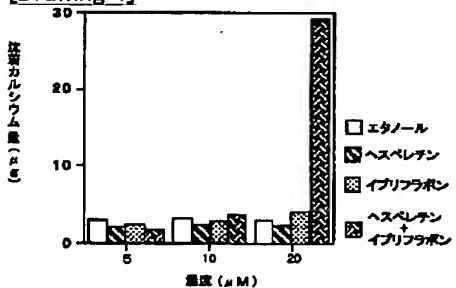
[Drawing 2]



[Drawing 3]



[Drawing 4]



[Translation done.]